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THE MULTIPLE TESTIS IN URODELES.

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In a recent paper ("The Interstitial Cells of the Urodele Testis," 1921) the writer referred briefly to the occurrence, in various urodeles, of what might be termed a multiple testis—a testis made up of a series of enlargements, each of which is morphologically and functionally similar to the others or to the simple testis found in the majority of American urodeles. Successive enlargements are separated by constricted regions, often of greater length than the enlargements themselves. Such regions have been referred to by investigators as "sterile." This designation, however, is clearly inapplicable, since, as we shall see, reproductive cells are not absent.

Of the twelve species of American urodeles examined by the writer, multiple testes are found to occur in three—*Desmognathus fusca*, *Diemyctylus viridescens*, and *Diemyctylus torosus*.¹ Among the urodeles of Europe, according to Champy, axolotl and the tritons exhibit this feature. I myself have examined testes of the European *Salamandra atra* and find them to consist of as many as three enlargements or lobes.²

Though the multiple testis is of rather common occurrence, no satisfactory discussion of its origin and significance has yet been encountered in the literature. Following are a few of the most

¹ von Wittich ('53) describes and illustrates (his Fig. 18) the testis of *Necturus lateralis* (*Necturus maculosa*) as consisting of three parts or lobes. Hoffmann ('73-'78) in Bronn's "Klassen und Ordnungen der Amphibien", states that von Wittich also describes the testis of *Menopomā* (*Cryptobranchus alleghehiensis*) as of the multiple type. The author has found in *Necturus* no testes such as that illustrated by von Wittich, though about sixty males have been examined; neither has a multiple testis been found in any of the six *Cryptobranchus* males studied.

² Each enlargement or secondary testis will in this discussion be termed a lobe, a designation agreeing with that employed by Champy and other workers.

pertinent comments: Kingsbury ('02) noted the occurrence of lobes in the testis of *Desmognathus*. He says: "There seems to be no absolute correlation of this condition with other structural features of the salamander, save that the presence of two or more enlargements occurs more often—in fact, quite constantly—in large animals. A similar division of the testis into 'lobes' occurs in other salamanders with an elongated body, and has been noted in *Amphiuma* and *Spelerpes*.¹ The segmented condition of the organ in *Cæcilians*² is perhaps to be associated likewise with the elongated form of the body."

Champy, similarly, comments on the relation of body size to the presence of the multiple type of testis. He believes that the variation in the number of lobes is correlated with that in the size of the species. He found the lobes very numerous in axolotl, more numerous in *Triton cristatus* than in *Triton palmatus* and *Triton punctatus*, and least numerous in the salamanders, though the last-named animals, he concedes, are of rather large size. Champy further considers that among the individuals of any species the number of lobes will be proportional to the size of the animal. He states that animals reared with insufficient food remain small and their testes develop few lobes, while an animal of one year, well nourished, may possess a testis of numerous lobes.

From my own observations on the testes of numerous species of American urodeles it would appear that the general conclusions stated by previous workers are in part inapplicable. The size of the species appears to be in no way correlated with the occurrence of a multiple testis. For example, our largest species, *Cryptobranchus* and *Necturus*, have testes of the simple type; three species of medium size—*Plethodon glutinosus*, *Gyrinophilus porphyriticus*, and *Amblystoma punctatum*—likewise have testes of unit

¹ The writer is unable to confirm the occurrence of a multiple testis in *Spelerpes*, though about a dozen males of the species *Spelerpes bislineatus* have been examined. McGregor ('99) likewise states that no division of the testis into lobes occurs in *Amphiuma*.

² The multiple testes of the Cæcilians, judging from the descriptions available, are probably of a type different from those of the Urodeles. Spengel ('76) describes the enlargements as being numerous in an immature animal, and states that they are separated by regions from which germ cells are entirely absent. Urodele males show no such primary growth centers; neither are germ cells entirely absent from any region of the testis.

structure. The species possessing the multiple testes (*Desmognathus* and *Diemyctylus*) are, in comparison with the above, small animals, yet their testes may consist of from two to five lobes. Plainly the size of the species is not to be regarded as a factor.

That the elongated form of the body is responsible for the multiple testis is likewise hardly conceivable. Among our American urodeles none are more slender and graceful of body than *Gyrinophilus* and *Spelerpes bislineatus*. Yet in these animals the testis, though an elongated structure, is not divided into lobes. *Desmognathus* and *Diemyctylus*, possessing multiple testes, are comparatively stout-bodied animals.

Champy's conclusion that the number of lobes in males of the same species is proportional to their body length is only in a limited sense correct, as the following figures from my records show: The average length of eight *Desmognathus* males with testes of two lobes each is 9.8 cm.; the average length of six males with testes of three lobes is 10.2 cm. or 0.4 cm. greater. Nevertheless one of the individuals of the first group measured 12.2 cm., while no animal of the second group exceeded a length of 10.5 cm. The average body length for the same group of eight males having testes of two lobes each is 9.8 cm. Within this group, however, are lengths ranging from 8 cm. to 12.2 cm. Clearly other factors than mere body length must be concerned, else we should find much less variation within this group.

Conceding that males which have been kept small by lack of food may develop testes of few lobes, as Champy states, the writer is forced, so far as *Desmognathus* is concerned, to doubt Champy's further claim that animals well fed may become very large, and, at one year of age, possess testes of numerous lobes. Since the urodele male completes but one spermatogenetic cycle annually, the males of one year referred to by Champy would of necessity reach sexual maturity with testes of many lobes already developed. In other words, the multiple testis, whatever its correlations, would develop simply through the initiation of greater growth activity in localized regions of the original cord of germ cells, as suggested by Kingsbury ('02). A male approaching sexual maturity might, on the basis of this theory, possess an even greater number of lobes than one which had been sexually active for several seasons.

Champy, indeed, states his belief that the number of lobes is not at all dependent upon the age of the male.¹ In *Desmognathus*, however, the evidence shows clearly that the sexually immature male, regardless of size and vigor, possesses only a simple testis—*i.e.*, only one lobe is present. The multiple testis, then, must arise by the addition of other lobes through growth processes *following sexual maturity*; in other words, the *age* of the animal must be considered as a factor.

Preceding investigators, so far as can be ascertained, have merely noted the occurrence of the multiple testis and speculated as to its possible correlations. The origin of the numerous enlargements or lobes has not been adequately investigated, and it is to their origin and development that we must turn if we are to fully appreciate their significance. Spengel ('76) makes the brief statement that the numerous lobes of a multiple testis arise as the result of the complex growth, degeneration, and regeneration processes of the organ.² This statement, properly elaborated, furnishes, I believe, the correct interpretation of these structures. The pattern of the spermatogenetic processes, in other words, explains the multiple testis. It is clearly not a structure of segmental origin; its correlation with the body size or body form of the species or the individual of the species can not be established. It is, as I shall attempt to show by a discussion of the urodele plan of spermatogenesis, a structure arising from the combined operation of three factors: (1) the slow movement of the spermatogenetic "wave"; (2) the delayed regeneration of the emptied lobules at the close of the spermatogenetic cycle; (3) the age of the animal—*i.e.*, the number of sexual cycles through which the two first-named factors have been operative.

¹ An earlier French worker, Duvernoy ('51), according to Spengel, reached a similar conclusion. Spengel (p. 65) says: "Er glaubt, die Zahl der Abschnitte sei allein abhängig von der Brunst, da er keine constanten Altersunterschiede zu entdecken vermochte."

² Spengel (p. 65) says: "Die eingehendere Erörterung dieser Frage muss ich bis zur Darstellung der Entwicklung und des Wachstums verschieben . . ." Whether Spengel ever published a more detailed explanation of the multiple testis, as the foregoing quotation shows he proposed doing, I am unable to state. No reference to any later work by him on the amphibian testis has been encountered in the literature, although his first publication, "Der anatomische Bau des Urogenitalsystems," is often extensively quoted.

The structure of the urodele testis has been described at length in a previous communication and need be only briefly reviewed at this time. The elongated testis is suspended from the dorsal abdominal wall by a mesorchium in which run the blood vessels and efferent ductules. The latter, varying from few to several in number, lead from the longitudinal collecting duct of the testis to the ductus deferens (Wolffian duct). The longitudinal collecting duct may be superficial in position, or as in many urodeles, more centrally located. The structural units of the testis, the lobules,¹ empty into the longitudinal collecting duct either directly, by means of very short ducts, or more indirectly, by way of longer, much-branched tributary ducts.

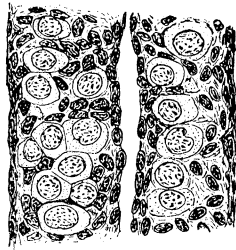


FIG. 1. Longitudinal section of the extreme anterior end (primary germ-cell cord) of a *Desmognathus* testis. The longitudinal collecting duct is shown, bordered by primary spermatogonia. The caudal germ-cell cord remaining after the complete degeneration of the lobules is essentially the same structure, as is also the slender cord intervening between successive lobes. The primary spermatogonia, in these latter structures, are properly termed "residual" spermatogonia.

A study of the lobule itself shows it to be a structure made up of reproductive cells all in approximately the same stage of development. Figure 2 shows the lobules as hollow cyst-like chambers lined by spermatogonia. All the cells in a lobule undergo their developmental transformations synchronously, mature as spermatozoa and leave the lobule. There remain behind only the Sertoli cells—which soon degenerate and disappear—and the residual spermatogonia. The latter are germ cells located only in the apex of the lobule, where it joins the collecting duct system.

¹ The term lobule, introduced by Kingsbury ('02), is used here in preference to the terms "cyst," "capsule," "tubule," etc., employed by various investigators.

Though present, they have remained quiescent during the months preceding the extrusion of the spermatozoa; with the emptying of the lobule, however, or even somewhat previously in many urodeles,

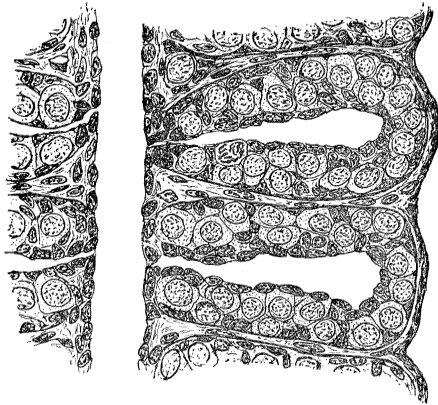


FIG. 2. Longitudinal section of a *Desmognathus* testis, showing a region somewhat posterior to that pictured in Fig. 1. Developing lobules are here well defined and their relation to the collecting duct clearly indicated.

they begin to multiply. By increase in their numbers the emptied lobule is in time completely regenerated; its germ cells again come to maturity and the cycle of degeneration and regeneration is repeated. That a delay in the regeneration of a group of emptied lobules would appreciably modify the form of the testis is self-evident. Such delayed lobule regeneration, as a factor in the formation of multiple testes, will be referred to subsequently.

All the lobules of the urodele testis do not, as a rule, mature and discharge spermatozoa at the same time. Examination of the testis shows the older developmental stages of the germ cells to be localized in its caudal region, while the earlier or younger stages are found more cephalically. In the course of spermatogenesis a wave of developmental change passes from the caudal to the cephalic end of the organ. The lobules of any region, therefore, will contain reproductive elements similarly advanced in development; caudal to any such region older stages are encountered while anterior to it are successively younger stages. Finally, at the anterior end of the testis will be found a slender cord or filament (see Fig. 1) containing only primary spermatogonia.

The caudocephalic movement of the spermatogenetic processes, or, as it has been appropriately termed, the spermatogenetic wave, varies greatly in rate with the species of urodele considered. In *Amblystoma* and *Cryptobranchus* it is comparatively rapid. As a result all regions of the testis tend to be in approximately the same stage of development at any particular time. In other urodeles, such as *Necturus* or *Gyrinophilus*, the rate is considerably slower, and in these several developmental stages will usually be encountered in cephalocaudal succession. Finally, in *Desmognathus* and *Diemyctylus* the rate is extremely slow, the spermatogenetic wave traversing only a part of the length of the testis in any one sexual cycle.¹ At a certain period in the cycle the secondary spermatogonia toward the anterior end of the organ cease to transform into spermatocytes I. A few lobules of these spermatogonia may degenerate entirely or in part, as described by Kingsbury and Hirsh ('12). These degenerated lobules mark a "boundary plane" caudal to which, in the current season, all the reproductive cells are matured as spermatozoa. Anterior to this boundary plane the germ cells are held over until the following season, when the wave resumes its forward movement from the point at which development was checked in the preceding season.

The testis of *Desmognathus*, then, is characterized by a slow movement of the spermatogenetic wave. Consider, now, the action of this factor, in connection with delayed lobule regeneration, as to possible effect on the structure of the testis. The testis of *Plethodon*, which is always of unit structure, may be used for comparison with that of *Desmognathus*, since the two agree in possessing a central² longitudinal collecting duct round which the lobules are arranged as are the spokes of a wheel. The two are in contrast, however, both as to the rate of the spermatogenetic wave and the rapidity of lobule regeneration, after extrusion of the spermatozoa. In *Desmognathus* both processes go on slowly; in *Plethodon* both proceed with comparative rapidity, especially the processes of lobule regeneration.

¹ The urodele male, it will be recalled, completes but one sexual cycle annually.

² The collecting duct in *Desmognathus* may, however, be more or less superficial towards the ends of the enlargement or lobe.

In developing males of either *Desmognathus* or *Plethodon*, the testis, subsequent to the establishment of the urino-genital connection, consists of but a slender cord of primary spermatogonia much as in Fig. 1, along whose entire length extends the longitudinal collecting duct. So far the testes of the two animals are substantially alike. Neither shows any indication of the establishment of growth centers such as might result in the formation of a many-lobed organ. Preceding the onset of sexual maturity the spermatogonia of the caudal end of the germ cell cord begin division, and eventually fully developed lobules are formed. This development, of course, proceeds caudocephalically in both animals. In *Plethodon*, whose wave rate is the more rapid, lobules are developed and spermatozoa are matured, in the male's first year of sexual maturity, throughout a considerable part of the length of the original germ cell cord. In *Desmognathus*, because of the slower wave rate, spermatogenesis is checked after proceeding but a fraction of the length of the gonad. A boundary plane is established in the manner previously mentioned, and spermatozoa are matured only caudal to its location.

At the time of maturity of the first spermatozoa, then, the testes of the two animals agree in consisting of but one lobe each. That of *Plethodon* is represented diagrammatically by Fig. 24 of Chart I., in which the unshaded area represents the part maturing spermatozoa. The testis of the young *Desmognathus* male is represented by Fig. 1, in which the same scheme of shading is employed. The boundary plane is indicated by the line *b*. The smaller fraction of the testis becoming functional in *Desmognathus* is to be noted.

Following the extrusion of the spermatozoa, in *Plethodon*, lobule regeneration proceeds rapidly. (It begins, indeed, considerably before the spermatozoa leave the lobule.) Though the form of the organ, before all of the more anterior lobules have been emptied and regenerated, will appear as in Fig. 25, prompt regeneration of the lobules during the winter—the spermatozoa being extruded in autumn—brings the testis, by spring, back to the type shown in Fig. 24. The process is repeated in succeeding seasons with no essential variation, save that the spermatogenetic wave, as the male becomes older, travels each year over a greater extent of the anterior portion of the germ cell cord (represented in black in Fig. 24)

in which, during the first season of the male's sexual activity, the germ cells did not develop beyond the secondary spermatogonial stage. In this way the testis is increased in length somewhat from year to year as the animal develops.

In the *Desmognathus* male, however, the extrusion of the spermatozoa in autumn is *not* followed, as in *Plethodon*, by prompt regeneration of the emptied lobules. The few residual spermatogonia located at the apex of each lobule appear to remain quiescent for several months. During the winter the emptied lobules slowly degenerate. The connective tissue cells surrounding them hypertrophy, forming interstitial cells, as described in detail in a previous communication. In the following summer the testis appears as in Fig. 2 of Chart I. The region anterior to the boundary plane *b* in Fig. 1 has developed to form the functional testis, in which the forward movement of the spermatogenetic wave proceeds slowly until again checked at the new boundary plane *b'*. The region containing degenerating lobules and interstitial cells is shown posterior to *b* (cross-hatched). It is to be kept in mind, of course, that the longitudinal collecting duct, surrounded by scattered groups of residual spermatogonia, extends throughout this region. The degenerating lobules and interstitial cells gradually disappear, until finally only the collecting duct and residual spermatogonia remain. In Fig. 2*A* the testis is represented as seen late in the summer following the animal's first year of sexual activity. The extreme caudal part, reduced to a condition similar to that of the anterior cord of spermatogonia, may be referred to as the "caudal germ-cell cord". The functioning region of the testis shown in Fig. 2*A* empties, and the cycle of changes outlined above is repeated. The caudal germ-cell cord, in this way, is increased in length, as shown in Fig. 3. This figure represents the testis of a male as in the summer after the second extrusion of spermatozoa. The positions of the boundary planes of successive seasons are indicated by *b*, *b'*, and *b''*.

Finally, after several months of inactivity, the residual spermatogonia in the most posterior part of the caudal germ-cell cord begin to multiply. Their multiplication leads to the formation of lobules of secondary spermatogonia; these in due season become spermatocytes I. and finally mature as spermatozoa. The testis,

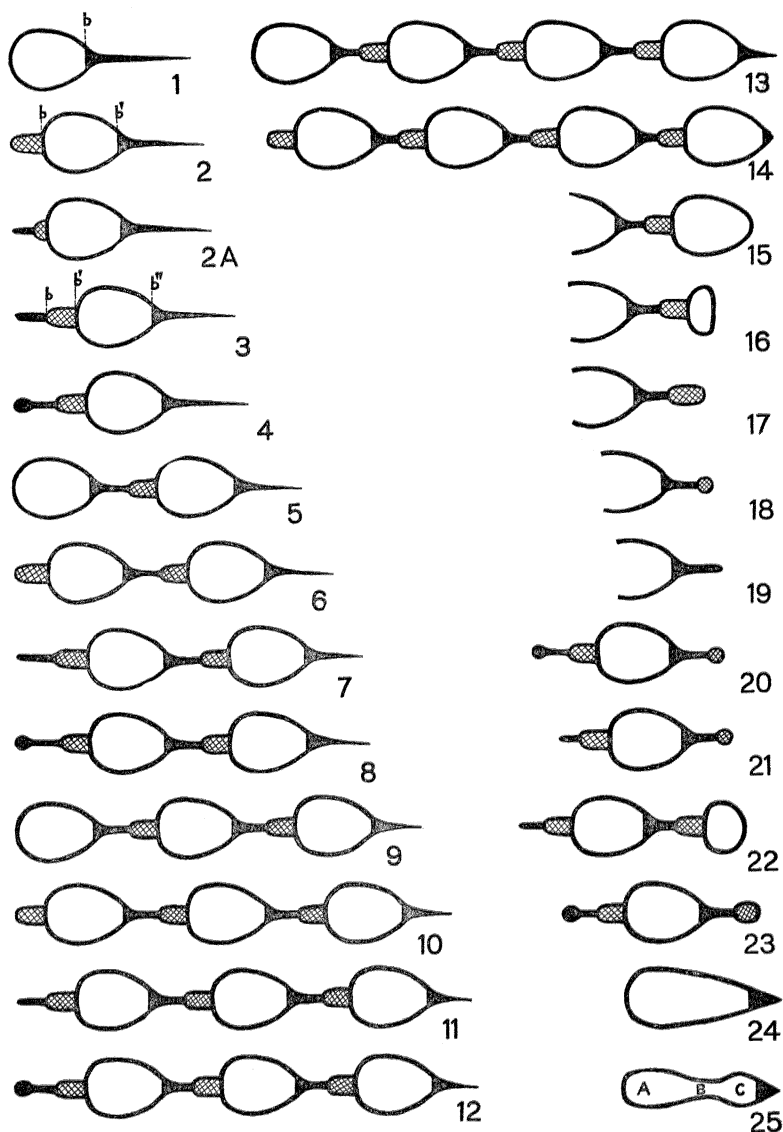


CHART I. FIGS. 1 TO 14. Diagrams illustrating the origin and development of the multiple testis of *Desmognathus*, up to a four-lobed stage. See Tables I. and II. for average lengths of animals having testes of these types. The cephalic end of the testis is at the right in all these figures. Solid black represents regions occupied by primary or secondary spermatogonia. Unshaded areas are those occupied by later stages of the germ cells—spermatocytes I. to mature spermatozoa. Cross-hatched areas are

at the time the lobules first begin regeneration, appears as in Fig. 4. A completely developed second lobe is represented in Fig. 5.

The spermatogenetic wave moves slowly cephalad along the caudal germ-cell cord in the same fashion as it moved along the primary germ-cell cord in the case of the first lobe. Boundary planes are established, in each season, in the same manner; anterior to these the transformation of the spermatogonia into spermatocytes I. ceases. In other words, a second "testis" has been established, caudal to the first and separated from it by the so-called sterile region—a region sterile only in the sense that its germ cells are temporarily inactive. This "testis" or lobe shifts forward on the trail of the first, leaving behind it, when first it empties, a caudal degenerating region such as is represented in Fig. 6. By the disappearance of the degenerating lobules and interstitial cells,

occupied by degenerating lobules and interstitial cells. Fig. 2*A* represents the testis as seen in later summer; all other diagrams are as of testes in June after the boundary plane (*b*, *b'*, etc.) for the season's development of spermatocytes has been established.

FIGS. 15 TO 19. Diagrams of the cephalic portion of the testis of *Desmognathus*, illustrating the disappearance by "running out" of an anterior lobe. Regions of the testis are represented as in the preceding figures. Figs. 15 and 16, testes as in June. Fig. 17, the same testis as in Fig. 15 or 16, but in June one year later. Fig. 18, the same testis later in the summer, prior to the final disappearance of the lobe. Fig. 19, the same testis in September after the last trace of the lobe has disappeared.

FIGS. 20 AND 21. Diagrams of multiple testes of *Desmognathus* approaching a simple type by reduction of the number of lobes.

FIGS. 22 AND 23. Diagrams indicating the differences observed between the testes of an individual. One testis (Fig. 23) is a season ahead of the other in development, as indicated by the earlier disappearance of the anterior lobe and the earlier development of a new lobe caudally.

FIG. 24. Testis of *Plethodon* male for comparison with Fig. 1. Represented as in midsummer. Note the absence of a long anterior germ-cell cord.

FIG. 25. The testis of Fig. 24 as in the fall, some time after extrusion of the spermatozoa had begun. Region *A* contains developing lobules of secondary spermatogonia for the next sexual cycle. Region *B* contains less well-developed lobules, and many degenerating lobules and interstitial cells. In region *C* the lobules still contain spermatozoa, but the lobules of the next cycle are already beginning their development. Black in this and in Fig. 24 indicates the part of the testis not maturing spermatozoa in the first season; i.e., a region containing only primary and secondary spermatogonia. Fig. 25 illustrates the nearest approach to lobation in the testis of *Plethodon*; the rapid development of lobules in regions *B* and *C* soon restores the testis to the type of Fig. 24.

from this region, a caudal germ-cell cord is again established as in Fig. 7; at the posterior end of this cord, eventually, lobule regeneration begins and a third lobe (see Figs. 8 and 9) is produced in exactly the same way as was the second. In this way multiple testes of as many as five lobes may come into being, though this number, I may add, has been found in but one specimen. Likewise, but one specimen with testes of four well-developed lobes has been noted.

The exact time interval that elapses between the first extrusion of spermatozoa and the regeneration of the region they occupied as a caudal second lobe has not been stated in the above description. As lobules emptied in autumn degenerate very slowly, and the interstitial cells marking their former location persist even until the following autumn, the time interval before the emptied region is completely reduced to a simple germ-cell cord is approximately a year. The spermatogonia in this cord, it appears, do not begin their development at any fixed time after the final disappearance of the degenerated lobules, but may remain quiescent for several months longer. Testes similar to those in Figs. 3 and 4 have been taken from specimens killed in June. Judging from the length of the caudal germ-cell cord as compared with that of the functioning testis, the formation of the second lobe could not be considered as having taken place in less than sixteen to eighteen months after the region first matured germ cells. Since the tiny caudal lobes found in June or July will contain only spermatogonia during the current season, they will not ripen spermatozoa until the end of the following summer. In short, the second lobe of a multiple testis may possibly come to full functional maturity only after an interval of three years from the time its territory of the testis first matured sex cells—that is, in the animal's third season of sexual activity. Since after the disappearance of the degenerating lobules and interstitial cells there is no means of distinguishing regions in the caudal germ-cell cord, it is doubtful whether the exact time interval before lobule regeneration begins can be determined. It would appear, indeed, that this time varies in different animals, since in males killed at the same time, with testes having small second lobes developing, these lobes will vary considerably in degree of development; though, doubtless, they are all to be re-

ferred to regions of the testis emptying in the same autumn, in some animals the regeneration of the lobules has been more delayed than in others. It is doubtful, too, whether a caudal lobe, when it develops, occupies exactly the same extent of the germ-cell cord as was occupied by the lobe which preceded it. Caudal lobes of animals killed in September or October may show only a half dozen lobules of mature spermatozoa, or they may be, on the other hand, almost or quite equal in size to the lobes anterior to them. The balance of metabolic processes in the animal doubtless determines the extent of movement of the spermatogenetic wave in any season; hence its cephalic progress in a caudally developing lobe may be checked and the size of the lobe limited quite independently of the exact time that had elapsed since the region was first emptied. The caudal lobe of Fig. 5, therefore, is not necessarily the exact equivalent, in point of territory occupied, of the testis of Fig. 1, but may occupy a greater or less extent of the germ-cell cord.

In each year of sexual activity, as has been stated, the most anterior lobe of the testis is shifted forward by exactly the extent of the region maturing and extruding spermatozoa. It is inevitable that eventually this anterior lobe will reach the cephalic end of the anterior germ-cell cord. By the time this occurs, however, there will be one or more functional lobes established posterior to it. Hence the animal's sexual activity is in no way interfered with by the final disappearance of an anterior lobe.

Such a disappearance or "running out" may be as easily demonstrated as is the caudal addition of new lobes. In Fig. 15 is shown an anterior lobe cephalad of which there is no cord of primary or secondary spermatogonia. This condition marks the first stage in the disappearance of the lobe. In Fig. 16 is represented a lobe somewhat similar to the above, save that it is much shorter; evidently, in the preceding year, only a comparatively small number of lobules of secondary spermatogonia had remained ahead of the boundary plane when it was established, and had been held over for later development. In Fig. 17 a more advanced stage in the running out of a lobe is represented. Here the most anterior lobules of the germ-cell cord have matured and extruded spermatozoa, and are found undergoing degenerative changes, sur-

rounded by sheaths of interstitial cells. Testes of the type shown in Figs. 15 and 16 are frequently encountered in June or July. In another year the testis of Fig. 15 would reach the stage shown in Fig. 17. Then, by the disappearance of the degenerating lobules and interstitial cells during the summer and early fall, all trace of the lobe finally vanishes, as illustrated in Figs. 18 and 19, and only a slender germ-cell cord remains.

The number of lobes that may possibly be formed, then, is limited by the extent of the primary germ-cell cord. This may and doubtless does increase by growth in length of its anterior portion as the animal develops. In any event, it varies considerably in different males, since the anterior lobe may appear as in Fig. 16 or 17 even when only two lobes are present, or it may show no indication of running out in a testis of from three to four lobes. An unusually long germ-cell cord permits the development of numerous lobes; a short cord reduces the number, though in no case has it been apparent that a very large old *Desmognathus* male possessed but a single lobe. Animals have been found, however, with multiple testes (as in Fig. 20) consisting of one large functional lobe, a very small lobe developing caudally, and a trace of an anterior lobe. Similar to these, but one step nearer to a simple testis, is that represented in Fig. 21. In this case no caudal lobe has yet begun its growth, and the anterior lobe is reduced almost to total disappearance. This type of testis in *Desmognathus* is exceptional, only one having been encountered in an examination of over a hundred males.

From the preceding description it is readily seen that a combination of two factors is essential in the production of the multiple testis of *Desmognathus*. These two factors, let me repeat, are the slow movement of the spermatogenetic wave and the delayed regeneration of the emptied lobules. Neither of them alone could give rise to a multiple testis. If, for example, the slow spermatogenetic wave is combined with rapid regeneration of the lobules, the length of the testis is merely increased each year by additions from the anterior germ-cell cord, but remains a unit, as in the *Plethodons*. If, on the other hand, a rapid spermatogenetic wave passed the complete length of the testis in a single season, as in *Cryptobranchus*, but lobule regeneration were then delayed for the

length of time that it is in *Desmognathus*, the animal would, of necessity, pass an interval of one or more years of sexual inactivity before reproduction again became possible. Such a condition, of course, does not exist in any known urodele.

Even in an animal possessing the spermatogenetic pattern requisite for the production of a multiple testis it is further essential that considerable time elapse after sexual maturity before a second testicular lobe is developed, and, following this, still another interval before the appearance of a third. All the evidence from *Desmognathus* is contradictory to Champy's assertion that age is not a factor, and that a male one year of age may possess testes of several lobes. Sexually immature males with more than one lobe are unknown to the writer. Large, heavy-bodied males invariably have multiple testes; these males, however, are always sexually mature, as shown either by the presence of spermatozoa or the occurrence in the testis of degenerating lobules emptied in a previous sexual cycle. Further, these large, heavy-bodied males, when one takes them in large numbers from their natural environment, must also average *older* than slender-bodied males of half to two thirds their body length taken from the same environment. Males of this last class average a smaller number of testicular lobes. *In short, the younger the animal, the smaller the number of lobes that will be found to have developed.*

As previously stated, the writer does not consider that the number of lobes is associated with body size alone, as claimed by Champy. Since, however, body size is to a certain extent correlated with age, an incidental correlation between lobe number and body length may be expected as a result. Examination of *Desmognathus* males shows some sexually mature animals to be only of 6 to 7 cm. total length, while the largest males measure 10 to 12 cm. A graphic representation of the relation of size to the form of the testis is presented in chart II. For graph *A*, stages 1 to 14 in the development of a multiple testis, taken from chart I., are used as abscissæ, and the corresponding average body lengths of the males, as given in Table I., are used as ordinates. It will be noted that up to stage 5 there is a fairly gradual increase in body length, along with the increase in the number of lobes. Animals at this stage—

TABLE I.

LENGTHS OF DESMOGNATHUS MALES FROM STREAMS ON TURKEY HILL,
NEAR ITHACA.

Arranged in groups on the basis of the type of testis. These types, listed in first column, correspond in number with the types illustrated in chart I. The one animal with testis of five lobes is not included in this table; since it had suffered the loss of a portion of its tail, its length could only be estimated.

Testis Type No.	Number of Lobes.	Number of Specimens Examined.	Length in Centimeters.		
			Maximum in Group.	Minimum in Group.	Average of Group.
1	1	4	5.8	5.0	5.4 -
2	1 +	11	8.0	5.5	6.7 +
3	1 +	10	9.5	7.3	8.3 -
4	2	18	10.2	8.0	8.6 +
5	2	8	12.2	8.0	9.8 -
6	2 +	16	12.2	8.0	9.8 +
7	2 +	6	11.2	8.0	10.2 -
8	3	5	10.7	10.0	10.3
9	3	4	12.0	9.0	10.6 -
10	3 +	6	10.5	9.7	10.2 -
11	3 +	0	—	—	—
12	4	0	—	—	—
13	4	1	11.0	11.0	11.0
14	4 +	2	12.0	10.5	11.2 +

that is, with testes of two complete lobes—have reached practically their full size, since after this, if we disregard the three animals representing stages 13 and 14, there is but slight upward or downward shifting in the level of the curve. Hence the maximal size may be attained when the testis is at stages 5 to 9, but the number of lobes may continue to increase for some time before checked by the running out of the anterior lobe at the cephalic end of the germ-cell cord. If the correlation were merely one of size rather than of age, one would expect a curve of quite a different type—a curve continuing upward in the same direction as the portion 1 to 5. This portion of the curve, incidentally, shows the correlation of size and lobe number in the sexually mature but growing male; after full size is attained lobe number still increases, and the last half of the curve maintains a rather constant level.

Graph A is based upon animals taken largely from streams on Turkey Hill, about three miles from Ithaca. The animals of these

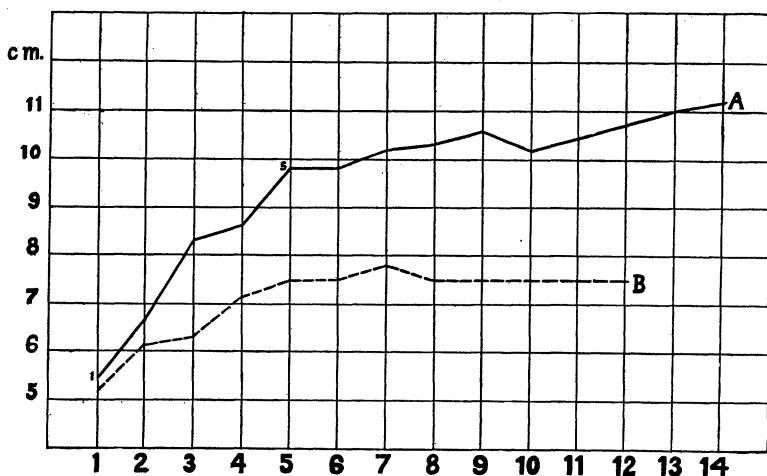


CHART 2. Graph *A* illustrates the relation of testis structure to size in the 93 *Desmognathus* males listed in Table I. The structural types from the first column of this table (see chart 1, Figs. 1 to 14) are here used as abscissæ, and the corresponding average lengths from the last column are used as ordinates. Between stages 5 and 14 an increase in length of but 1.2 cm. is indicated; the number of lobes of the testis increases with age, while the body size, after a certain period, remains relatively fixed. Between stages 1 and 5, on the other hand, there is an increase in length of 4.4 cm. Males mature spermatozoa long before they have attained their maximal size; the processes that operate to produce a multiple testis begin at sexual maturity; hence the development of the first additional lobe is accompanied by the continued increase in body length.

Graph *B*, constructed in the same manner as Graph *A*, shows the relation of testis structure to size in the 24 animals listed in Table II. Between stages 5 and 12 there is here a variation of only 0.3 cm. in average length, as compared with the increase of 1.4 cm. between stages 2 and 5. These animals average much smaller than the animals of the first group, as may be readily seen from the relative heights of the curves at any point. Running out of anterior lobes occurs relatively early and comparatively few lobes are developed; note that stages 9 to 14, represented by 13 males in Table I., have but one representative in Table II.

streams are the largest found in the vicinity of Ithaca, and are uniformly of a lightly colored type. Animals taken from the various gorges nearer Ithaca are ordinarily of smaller size and darker coloration. These have been grouped separately and a second graph (*B*) constructed which agrees in general with the first. For data concerning these animals, see Table II. The animals in this

group, as will be seen, are of much smaller average size and do not develop as many lobes as do the animals of the first group.

The multiple testis being purely the expression of the spermatogenetic wave rate and the time required for lobule regeneration, it follows that conditions modifying these factors will through them modify the form of the testis. Favorable metabolic conditions may increase to some extent the wave rate. The wave will travel farther in a season—*i.e.*, a greater number of lobules of secondary spermatogonia will develop into spermatocytes I. before such development is checked for the season and a boundary plane established. As a result the functional lobe will be of greater length than had the wave rate been slower. Lobes of large vigorous males, it is found, tend to be longer than those of smaller specimens. Greater lobe length, in turn, acts to reduce the number of lobes that may develop from a germ-cell cord of given length. Increase of the spermatogenetic wave rate beyond a certain limit, clearly, would reduce the number of lobes to one, and a multiple testis would not be developed.

Unfavorable metabolic conditions, similarly, may still further reduce the normally slow wave rate of *Desmognathus*. This would

TABLE II.

LENGTHS OF DESMOGNATHUS MALES OF SMALL DARK TYPE, TAKEN FROM GORGES NEAR ITHACA (Largely from Fall Creek Gorge on Cornell University Campus.)

Arranged in groups on the basis of the type of testis. The type numbers in the first column correspond to the type numbers of the diagrams of chart I.

Testis Type No.	Number of Lobes.	Number of Specimens Examined.	Length in Centimeters.		
			Maximum in Group.	Minimum in Group.	Average of Group.
I	I	I	5.2	5.2	5.2
2	I +	I	6.1	6.1	6.1
3	I +	5	6.9	5.5	6.3
4	2	5	8.1	6.1	7.1
5	2	2	7.2	7.7	7.5
6	2 +	5	8.2	6.0	7.5
7	2 +	3	8.0	7.5	7.8
8	3	2	8.0	7.0	7.5
9, 10, 11	3 +	0	—	—	—
12	4	I	7.5	7.5	7.5
13, 14	4 and 4 +	0	—	—	—

result in the formation of shorter lobes and permit the establishment of a greater number before a cephalic running out of the first lobe occurred. It seems probable that the presence of several lobes is of itself a factor in reducing wave rate. When a large number of lobes is present, each lobe is shorter, as a rule, than those of males with a smaller number. This acts, of course, in a full-grown animal, to keep the total functioning volume of the testis a constant as new lobes are added.

In the preceding consideration of the effect of wave rate it has been assumed that the time elapsing before lobule regeneration occurs has not been affected. This, however, is probably subject to the influence of metabolic conditions in the same way as is wave rate. We may safely assume, I believe, that lobule regeneration could be somewhat hastened by very favorable conditions or delayed by unfavorable ones. The effect in the first case would be to reduce the length of the interval between lobes, and therefore permit the establishment of a greater number; the effect in the second case would be of the opposite nature.

Favorable metabolic conditions, therefore, would tend to increase the wave rate and through it the size of the lobes, reducing at the same time their possible number; the same conditions, however, would probably cause earlier regeneration, shorten the interval between lobes, and tend thus to permit a greater number. Unfavorable conditions, by slowing the wave rate, shorten the lobes and thus increase the number possible; the same conditions, though, would tend to delay lobule regeneration and the formation of new lobes, increase the interval between lobes, and reduce the number. The net change, so far as the functional volume of the testis is concerned, is small; and excessive increase or reduction of the total output of reproductive cells will not occur, in a fully developed male, except under very unusual conditions.

The writer, therefore, though agreeing that bodily vigor may influence lobe number, insists that it does so only by affecting the length of the primary germ-cell cord, modifying the rate of the spermatogenetic wave, or changing the time necessary for lobule regeneration. In other words, given a spermatogenetic pattern such that multiple testes are possible, the modification of this pattern, by whatever influences it may be brought about, inevitably

modifies to some extent the end result. Hence one may expect a certain variation in lobe number even in animals of exactly the same age.

So, too, on this basis, the occasional differences between the testes of the individual male become understandable. Figures 22 and 23 illustrate the testes from animal number 437, killed on June 26. One testis (Fig. 22) shows a well-developed anterior lobe. In the other (Fig. 23) the disappearance of this lobe is practically complete. Nevertheless the almost microscopic enlargement containing degenerating lobules and interstitial cells shows that a functional lobe occupied this region in the preceding summer. At the caudal end of the testis represented in Fig. 23 a small lobe is developing; in the other (Fig. 22) only a slender caudal germ-cell cord appears. Clearly the one testis is a season in advance of the other, as evidenced both by the earlier disappearance of a lobe anteriorly and the earlier development of a new lobe caudally. Whether in the animal's first year of sexual maturity only one testis had become functional, or whether at some time later the spermatogenetic processes were retarded in one organ, it is impossible to state. Other animals have been found in which the two testes differ only in the size of the anterior lobes, they being of the types shown in Figs. 15 and 16. Still other males show differences only in the stage of development of the posterior lobes; animals are frequently found with one testis of the type shown in Fig. 4, while the other is as well developed as that in Fig. 5. Testes differing as do those of Figs. 5 and 6 have also been removed from a single individual. All the differences so far encountered, however, are but further evidence that the multiple testis is an expression of the spermatogenetic processes. Considering the lobes as structures of a segmental origin, or as divisions of the testis associated merely with the body size, the differences found in a single animal would be difficult of interpretation; realizing the manner in which lobes arise, run their course, and disappear, the occurrence of such differences is even to be expected. Though found in but a small number of the males studied, such differences are by no means rare; and, when found, they are to be looked upon as a normal result of the different modi-

fication in the two organs of those growth, degeneration, and regeneration processes which make a multiple testis possible.

In the writer's opinion, no particular phylogenetic significance attaches to this peculiar type of testis. It occurs in several members of the family Salamandridæ; Champy mentions it in numerous European tritons and salamanders, and I myself have observed it in our two American members of the family. If axolotl, on the other hand, possesses a multiple testis, as Champy states, it differs from its near relative, *Amblystoma punctatum*.¹ Among the Plethodontidæ, too, *Desmognathus* stands alone, no other member of the family, to my knowledge, possessing a multiple testis. Since the plan of spermatogenesis in *Necturus* or *Cryptobranchus*—in fact, in any of the urodeles I have examined—differs from that in *Desmognathus* or the Tritons in no fundamental way, a multiple testis might arise in any of these urodeles if the spermatogenetic processes became sufficiently reduced in rate. In this way, probably, have the species now possessing multiple testes arisen from ancestors with organs of the simple type. Such slowing of the reproductive processes in phylogeny might possibly be interpreted as due to deterioration in the vigor of the stock; the writer sees neither in it nor in the resulting structure of the testis any particular adaptative value.

SUMMARY AND CONCLUSIONS.

1. Multiple testes of two or more enlargements or lobes separated by intervening non-functional regions are of common occurrence in *Desmognathus* and other urodeles.

2. Such multiple testes are observed only in larger males. The smallest sexually mature males have testes of but a single lobe.

3. A study of the manner in which new lobes appear indicates the following mode of origin:

¹ Spengel says: "Beim Axolotl erscheint er (the testis) als eine breite, dicke, von zahlreichen Unebenheiten besetzte Platte." This description is somewhat more applicable to the testis of *Amblystoma punctatum*. Irregularities, however, are to be observed in the testis of *Amblystoma* only in the spring when the lobules are emptying, and are in no case so extreme as to be at all similar to the lobes of a true multiple testis. It may be questioned whether the "lobes" described by Champy in axolotl are similar in origin to those in *Desmognathus* and the tritons, or whether they are merely such "Unebenheiten" as Spengel mentions. No other investigator has described the testis in axolotl as being of the multiple type.

(a) The portion of the testis emptied in autumn is not regenerated immediately, since the residual spermatogonia remaining in the region do not begin division, but remain quiescent for several months.

(b) The functional testis in the next season develops anterior to the emptied region, from a part of the original germ-cell cord not developing spermatozoa in the first season.

(c) When the emptied lobules degenerate and disappear, the region they occupied remains as a slender strand or cord of residual spermatogonia.

(d) Spermatogonia in the caudal end of the cord finally begin division. Lobules of germ cells develop and a caudal "secondary testis" or lobe is formed.

(e) The second lobe shifts forward each season in the same manner as does the first. From the germ-cell cord left in its wake a third lobe eventually arises. The process is repeated in the case of other lobes subsequently added.

(f) The most anterior lobe finally reaches the cephalic end of the germ-cell cord and disappears. Other lobes follow in due time, but are replaced by new ones originating caudally.

4. New lobes have been found to appear in no other manner than by the process above outlined.

(a) Sexually immature males never show numerous growth centers in the germ-cell cord.

(b) New lobes do not arise by the promiscuous establishment of secondary growth centers between lobes already present.

(c) Small anterior lobes always prove to be disappearing or "running out"; new lobes have not been found to develop in this region.

5. The conditions necessary for the formation of a multiple testis are:

(a) A slow forward movement of the spermatogenetic wave.

(b) Delayed regeneration of the emptied lobules.

(c) The lapse of a time interval following which the long-postponed regeneration brings into existence the new lobe. The unregenerated region between lobes is that commonly termed "sterile."

6. The multiple testis is clearly but a result of the spermatogenetic pattern of the species possessing it.

7. Modifications of the spermatogenetic pattern cause differences in the number or size of the testicular lobes even in animals of the same age. Even in the individual the two organs, from a similar cause, may occasionally be of different type.

8. Neither the multiple testis nor the peculiar combination of factors producing it are regarded as of important phylogenetic or adaptative significance.¹

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